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CONTENTS

SECTION A.—PHYSICAL SCIENCES

Page

Rotational Structure of the Birge-Hopfield Bands of N_2 —*J. W. T. Spinks* - - - - - - - - - - - - 1

The Variances of the Means and the Variance of the Slope of the Line of Relation of a Linear, Composite, Bivariate Distribution—*F. Charnley* - - - - - - - - - 6

SECTION B.—CHEMICAL SCIENCES

Le Calcium et le Manganèse dans les Sèves et les Sirops d'Érable —*E. Bois, L. C. Dugal, et M. Lessard* - - - - - - - 1

Chemotherapeutic Studies: Preparation of Substituted Sulphonamides—*C. Marchant, C. C. Lucas, and L. McClelland*—with an Appendix—The Determination of Chemotherapeutic Activity—*P. H. Greey* - - - - - - - - - 5

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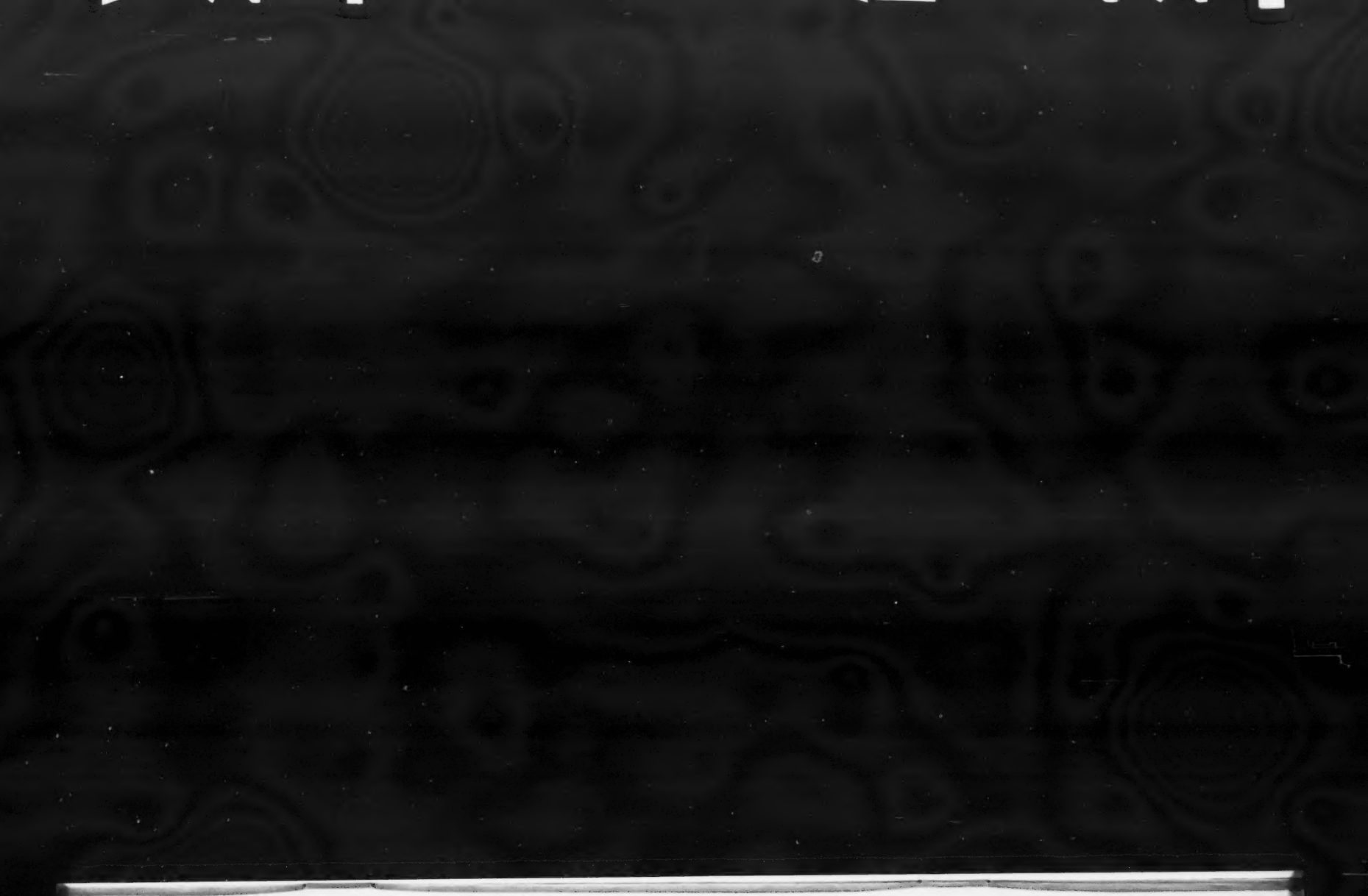

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ROTATIONAL STRUCTURE OF THE BIRGE-HOPFIELD BANDS OF N_2[1]

BY J. W. T. SPINKS[2]

Abstract

The 2–10, 3–11, 4–12, 5–13, 5–14, and 6–14 bands of the $a^1\Pi_u \rightarrow X^1\Sigma_g^+$ system in N_2 (Birge-Hopfield bands) have been photographed and measured. P, Q, and R branches are recorded and the indentification of the transition as $^1\Pi \rightarrow {}^1\Sigma$ is definitely confirmed. B', B'', α', and α'' values are presented.

Introduction

Molecular nitrogen has a strong system of bands in the Schumann region, and this system has been identified as a $^1\Pi_u - {}^1\Sigma_g^+$ transition (1, 2, 4). Such bands should show P, Q, and R branches, but, so far, measurements of R lines have not been recorded and, as a result, the certainty of the assignment of these bands to a $^1\Pi_u - {}^1\Sigma_g^+$ transition has been questioned.

The P, Q, and R branches in question showed up very clearly in some plates of the Birge-Hopfield system taken some time ago, and consequently it was thought worth while to record the results of measurements on these plates.

Experimental

Nitrogen from a cylinder was excited in a discharge tube of the type used for producing an intense hydrogen continuum. The photographs were taken in the third order of the 3-metre grating at the Physikalisches Institute in Darmstadt. Interference by the second order of the strong second positive group of nitrogen was eliminated by a semicylindrical quartz lens mounted in front of the slit of the spectrograph and used as a prism.

Hilger Schumann plates were used.

As usual the spectrum of the iron arc was employed as comparison spectrum and the plates were measured with an Abbé comparator.

Results

Rotational analyses of the 2–10, 3–11, 4–12, 5–13, 5–14, and 6–14 bands have been made. There is a good deal of overlapping in some of the bands, and it is usually impossible to separate the R branch lines in the head of the band. As would be expected, the branch lines show alternating intensities. The Q branch is much more strongly developed than the P and R branches,

[1] *Manuscript received October 14, 1941.*
Contribution from the Department of Chemistry, University of Saskatchewan, Saskatoon, Sask.

[2] *Professor of Physical Chemistry.*

and in most cases was measured to beyond $Q(40)$. Application of combination differences indicates that the Λ doubling in the Π state is very small. Assignments of line frequencies are given in Tables I, II, and III.

TABLE I

WAVE NUMBERS OF THE LINES IN THE $a^1\Pi_u \rightarrow X^1\Sigma_g^+$ N_2 BANDS

J	2–10 band. ν_0 = 50,252.50			3–11 band. ν_0 = 49,821.16		
	$Q(J)$	$P(J)$	$R(J)$	$Q(J)$	$P(J)$	$R(J)$
2	50,250.5	50,244.7+		49,820.4	49,813.6+	49.829.6+
3		39.5+				
4	47.5	35.2+		17.2+	04.2+	32.9+
5	44.7+		50,264.1+			
6	42.0	23.2+	64.1+	11.4	792.7+	32.9+
7	39.5+	16.7	64.1+	08.4+	85.4+	32.9+
8	35.2+	09.6		04.2+		32.9+
9	30.1+	01.9+		00.0	70.8+	
10	25.7	194.5+		795.1	63.4+	29.6+
11	20.5	86.6+		89.7	55.9+	27.1
12	14.7	77.6+	55.7	83.8	47.5+	24.6
13		67.3	51.8	77.9		
14	01.9+	57.6	48.8	70.8+	27.5	17.2+
15	194.5+	46.7	44.7+	63.4+	16.9	13.6+
16	86.6+	36.2		55.9+	06.4	08.4+
17	77.6+	24.9	35.2+	47.5+	695.4	04.2+
18	69.7	13.1	30.1+	39.2	83.4	797.6
19		00.5	23.2+	29.9		92.7+
20	50.8	87.9		20.1	58.0	85.4+
21	40.8	74.2		10.0	45.0	77.9
22	30.1	60.9		699.4	31.2	70.8+
23	19.2	46.8		88.1		63.4+
24	07.5	32.0	186.6+	76.7	02.8	53.9
25	95.5		77.7+	64.4	587.3	47.5+
26	82.7	01.2		51.9	71.8	35.5
27	69.3			38.8	56.0	25.4
28	56.3	49,968.6		25.5	38.9	13.9
29	42.3	51.0		11.2	22.0	03.2
30	27.8			597.1	04.5	692.4

+ *Refers to lines that are overlapped.*

TABLE II

WAVE NUMBERS OF THE LINES IN THE $a^1\Pi_u \rightarrow X^1\Sigma_g^+$ N_2 BANDS

J	4–12 band. ν_0 = 49,391.50			5–13 band. ν_0 = 48,964.05		
	$Q(J)$	$P(J)$	$R(J)$	$Q(J)$	$P(J)$	$R(J)$
2	49,390.1+	49,383.8+	49,398.5+	48,962.6+	48,956.5+	48,970.7+
3	88.2				52.1	
4	86.6+	74.1+	401.1+	59.0+	46.6+	
5	83.8+	69.2+		56.5+	41.7+	75.1+
6	80.8	62.6	02.8	53.9+	35.4	75.1+
7	77.8+			49.8+		
8	74.1+	47.5+	01.1+	46.6+	22.3	
9	69.2+	40.8+		41.7+	14.7	
10	64.8	33.5+	398.5+	37.3	07.0	70.7+

TABLE II—*Concluded*

WAVE NUMBERS OF THE LINES IN THE $a^1\Pi_u \leftarrow X^1\Sigma_g^+$ N_2 BANDS—*Concluded*

	4–12 band. ν_0 = 49,391.50			5–13 band. ν_0 = 48.964.05		
	$Q(J)$	$P(J)$	$R(J)$	$Q(J)$	$P(J)$	$R(J)$
11	49,359.7	49,325.8+	49,396.1	48,932.1+	48,898.3+	48,968.0
12	53.9+	17.2+	93.2	26.3+	90.0+	65.6
13	47.5+	08.6+	90.1+	19.8	81.2+	62.6+
14	40.8+	297.5	86.6+	13.1	70.8	59.0+
15	33.5+	86.3	83.8+	05.7	59.9	53.9+
16	25.8+	76.1	77.8+	898.3+	49.6	49.8+
17	17.2+	65.6		90.0+	38.3	43.9
18	08.6+	53.8	66.8	81.2+	26.9	38.7
19	299.6	41.2		72.2	14.5	32.1+
20	89.9	28.5	53.9+	62.4	01.8	26.3+
21	79.8	14.7	44.8	52.1	788.5	17.8
22	68.9	01.6	39.2	41.7	75.3	11.3
23	58.0	188.1	32.2	30.4	60.8	02.9
24	46.5	73.0	22.6	19.0	46.2	894.2
25	34.0	57.3		06.8	31.1	84.3
26	22.1	42.5	04.3	794.2	15.7	75.7
27				81.3	699.5	65.5
28				67.9	83.2	54.7
29				53.4	66.4	43.7
30				39.3	48.4	32.3

TABLE III

WAVE NUMBERS OF THE LINES IN THE $a^1\Pi_u \rightarrow X^1\Sigma_g^+$ N_2 BANDS

J	5–14 band. ν_0 = 47,009.30			6–14 band. ν_0 = 48,537.5		
	$Q(J)$	$P(J)$	$R(J)$	$Q(J)$	$P(J)$	$R(J)$
0						48,541.7
1						44.2
2	47,008.1+	47,002.6+		48,536.1		46.0
3						
4	04.7+	46,993.4				
5	02.6+	87.6	47,021.1+	30.8		49.2+
6	00.2+	81.9		27.7		49.2+
7	46,997.1	74.7+	21.1+	24.6		
8	93.4	69.2+		20.8	48,496.8	
9	89.0	62.6+		16.0	89.3	
10	84.9+	54.2	18.2	11.8	81.4	
11	79.5+	46.6	16.0	06.3		
12	74.7+	38.2	13.9	00.5		39.9
13	69.2	29.4	11.2	494.1		
14	62.6+	20.2	08.1+	87.5		
15	55.7	10.3	04.7+	80.2		
15	48.9	00.2	00.2+	72.7		
17	41.1	46,889.4	46,995.4	64.5		
18	33.3	78.7	90.4	55.3		
19	24.6	66.9	84.9+	46.0		
20	15.7	55.1	79.5+	37.2	48,377.0	
21	06.4	43.2	72.7	25.0		
22	896.6	29.9	66.1	15.2		
23	86.2	17.0	58.6			
24	75.6	02.8	50.9			
25	64.1	788.5				
26	52.7	73.8	34.0			

TABLE IV

COMBINATION DIFFERENCES, $\Delta_1F'(J)$, $v' = 5$ (UPPER STATE)

J	$Q(J+1) - P(J+1)$		$R(J) - Q(J)$	
	5–13 band	5–14 band	5–13 band	5–14 band
3	12.4+	11.3+		
4	14.8+	15.0+		
5	18.5+	18.3+	18.7+	
6		22.4+		
7	24.3+	24.2+		
8	26.9+	26.3+		
9	30.3	30.7+		
10	33.9+	32.9+	33.4+	33.2+
11	36.3+	36.5+	35.9+	36.4+
12	38.6+	39.7+	39.3+	39.2+
13	42.3	42.5+	42.8+	42.0+
14	45.9	45.4	45.9+	45.4+
15	48.6+	48.7	48.2+	49.0+
16	51.7+	51.6	51.5+	51.4+
17	54.3+	54.5	53.9+	54.4
18	57.8	57.7	57.5+	57.2
19	60.6	60.5	59.9+	60.3+
20	63.6	63.2	63.9+	62.8+
21	66.4	66.7	65.7	66.3
22	69.5	69.2	69.6	69.6
23	72.9	72.8	72.6	72.4
24	75.7	75.6	75.2	75.2
25	78.5	78.9	77.5	
26	81.8	81.5	81.5	81.3
27	84.7	84.1	84.1	
28	87.0	87.0	86.8	

+ *Using lines that overlap.*

The numbering was determined by the usual methods (see, for example, (3)). Table IV gives combination differences for the 5–13 and 5–14 bands. The agreement of the $\Delta_1F'(J)$ values indicates that the two bands have the same upper state. The fulfilment of the relation $Q(J+1) - P(J+1) = R(J) - Q(J)$ is illustrated in Table IV for the 5–13 and 5–14 bands and gives a check on the analysis of the bands. The rotational constants B_v were obtained from the combination differences in the usual manner, using Q–P differences. The rotational constant, D, was calculated from the equations below, using preliminary B's.

$$D_v = D_e + \beta(v + \tfrac{1}{2}): \quad D_e = \frac{-4B_e^3}{\omega_e^2}$$

$$\beta = \frac{\alpha^2}{6\omega_e} + D_e\left(\frac{8\omega_e X_e}{\omega_e} - \frac{5\alpha}{B_e}\right)$$

$$\omega_e'' = 2359.60: \quad \omega_e' = 1692.28$$

$$\omega_e X_e'' = 14.45: \quad \omega_e X_e' = 13.32$$

$$D_e' = -6.208 \times 10^{-6}: \quad \beta' = 9.54 \times 10^{-8}$$

$$D_e'' = -5.820 \times 10^{-6}: \quad \beta'' = 0.362 \times 10^{-8}$$

TABLE V

ROTATIONAL CONSTANTS

Band	Upper state, $a^1\Pi_u$		Lower state, $X^1\Sigma_g^+$	
	B'_v, cm.$^{-1}$	D'_v, cm.$^{-1}$(calc.)	B''_v, cm.$^{-1}$	D''_v, cm.$^{-1}$(calc.)
2–10	1.579	-5.97×10^{-6}	1.821	-5.78×10^{-6}
3–11	1.551	-5.87×10^{-6}	1.790	-5.78×10^{-6}
4–12	1.538	-5.78×10^{-6}	1.780	-5.78×10^{-6}
5–13	1.521	-5.68×10^{-6}	1.758	-5.77×10^{-6}
5–14	1.522	-5.68×10^{-6}	1.744	-5.77×10^{-6}
6–14	1.507	-5.58×10^{-6}	1.737	-5.77×10^{-6}

The B values are presented graphically in Fig. 1. The results of Appleyard and of Watson and Koontz (taken from their graph) are included. From the figure, $\alpha' = 0.022$ and $\alpha'' = 0.018$. The large number of R branch lines measured confirms the identification of the system as $a^1\Pi_u \rightarrow X^1\Sigma_g^+$.

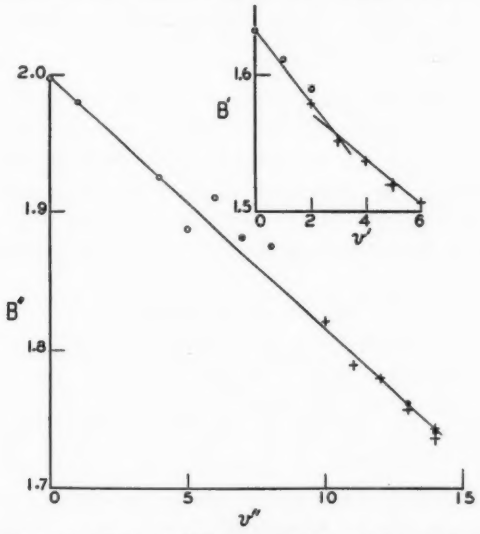

FIG. 1. *B_v vs. v curves for $a^1\Pi_u$ and $X^1\Sigma_g^+$ states of N_2: +, the writer's data; ●, Appleyard's data; ○, data of Watson and Koontz.*

Acknowledgments

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References

1. APPLEYARD, E. T. S. Phys. Rev. (Ser. 2) 41(2) : 254-255. 1932.
2. BIRGE, R. T. and HOPFIELD, J. J. Astrophys. J. 68 : 257-278. 1928.
3. HERZBERG, G. Molecular spectra and molecular structure. Prentice-Hall, Inc., New York 1939.
4. WATSON, W. W. and KOONTZ, P. G. Phys. Rev. (Ser. 2) 46(1) : 32-37. 1934.

THE VARIANCES OF THE MEANS AND THE VARIANCE OF THE SLOPE OF THE LINE OF RELATION OF A LINEAR, COMPOSITE, BIVARIATE DISTRIBUTION[1]

By F. Charnley[2]

Abstract

The variances of the means and the variance of the slope of the line of relation of a linear, composite, bivariate distribution, in which the variances and correlation coefficient of the component populations remain constant, are analogous to the corresponding variances of a linear regression line. The variances of the means are respectively, $\frac{\sigma_x^2}{N}$, $\frac{\sigma_y^2}{N}$, and the variance of the slope of the line of relation is $\frac{S_{YL}^2}{N(S_Y^2 - \sigma_x^2)}$, where σ_x^2, σ_y^2 are the variances of the component populations, S_{YL}^2 is the vertical variance of the composite distribution around the line of relation, N is the total number of measures, and S_Y^2 is the variance of the composite distribution around the Y-axis.

The variances of the means and the variance of the slope, m, of the line of relation of a linear, composite, bivariate distribution (1) that are needed in testing the significance of the differences in two lines of relation are entirely analogous to those of the corresponding parameters in a linear regression equation. In view of this close similarity the required variances might therefore be written down by inspection. They are, however, very easily deduced as follows.

Variances of Means

To find the variance in the mean, M_Y, of the composite distribution we need consider only the vertical deviations in the individual means of the component populations. The variance in the mean of a sample of n drawn from a single component population is $\frac{\sigma_y^2}{n}$. Hence, if there are k component populations and a sample of n is drawn from each component, the variance in the mean of the means of the samples will be

$$\frac{k\sigma_y^2}{k^2 n} = \frac{\sigma_y^2}{kn}.$$

If the n's differ, the variance will be

$$\sigma_y^2 \frac{\sum_1^k n_i}{\left(\sum_1^k n_i\right)^2} = \frac{\sigma_y^2}{\sum_1^k n_i}.$$

[1] *Manuscript received September 25, 1941.*

Contribution from the Canned Salmon Inspection Laboratory, Department of Fisheries, Vancouver, B.C.

[2] *Chief Chemist.*

Accordingly, in terms of single measures the variance in the mean of the Y-variate of a linear, composite, bivariate distribution is $\frac{\sigma_y^2}{N}$, where $nk = \Sigma n_i = N =$ the total number of measures. And similarly, the variance in the mean of the X-variate is $\frac{\sigma_x^2}{N}$.

Variance of Slope of Line of Relation

From the equation giving the value of m we have

$$m = \frac{\lambda'}{\lambda},$$

where λ' and λ are the respective standard deviations of the means around the means of the composite distribution. If δm is an error in m, then

$$\begin{aligned}\delta m &= \frac{\partial m}{\partial \lambda'}\,\delta\lambda' + \frac{\partial m}{\partial \lambda}\,\delta\lambda \\ &= \frac{\delta\lambda'}{\lambda} - \frac{\lambda'}{\lambda^2}\,\delta\lambda \\ &= \frac{1}{\lambda}\,(\delta\lambda' - m\delta\lambda).\end{aligned}$$

The square of the error in m is therefore

$$(\delta m)^2 = \frac{1}{\lambda^2}\left[(\delta\lambda')^2 - 2m\delta\lambda\delta\lambda' + m^2(\delta\lambda)^2\right].$$

Summing all the errors and dividing by the number of errors we thus obtain

$$\sigma_m^2 = \frac{1}{\lambda^2}\left[\sigma_{\lambda'}^2 - 2mr\sigma_\lambda\sigma_{\lambda'} + m^2\sigma_\lambda^2\right],$$

in which r is the correlation between the errors in λ and λ'.

Suppose now we draw samples of n from one population. We obtain means $y_{i1}, y_{i2}, \ldots . y_{ij}, \ldots$ These have errors $d_{i1}, d_{i2}, \ldots d_{ij}$. If y_i is the true mean of this population, the squares of the means of the samples are: $y_{i1}^2 = (y_i + d_{i1})^2$, $y_{i2}^2 = (y_i + d_{i2})^2 \ldots .$ The errors in the squares are therefore

$$\begin{aligned}\delta y_{i1}^2 &= y_{i1}^2 - y_i^2 = 2y_i d_{i1} + d_{i1}^2 \\ \delta y_{i2}^2 &= y_{i2}^2 - y_i^2 = 2y_i d_{i2} + d_{i2}^2, \text{ etc.}\end{aligned}$$

If $d_{i1}, d_{i2}, \ldots d_{ij} \ldots$, are small relative to the mean, y_i, which will be true if large samples of n are drawn from this population, $d_{i1}^2, d_{i2}^2, \ldots .$ may be neglected in comparison with $2y_i d_{i1}, 2y_i d_{i2}, \ldots .$ Accordingly, the variance of the square of the average of a sample of n is

$$\overline{(\delta y_i^2)^2} = 4y_i^2\bar{d}_i^2 = 4y_i^2\frac{\sigma_y^2}{n}.$$

But λ'^2 is the mean of the squares of k averages, each squared average having the variance $4y_i^2\frac{\sigma_y^2}{n}$, if the samples drawn from the individual populations

are each of size n. Hence, the variance of the sum of the squared averages is $4\sum_1^k y_i^2 \frac{\sigma_y^2}{n}$ and that of the mean of the squared averages

$$4\Sigma y_i^2 \frac{\sigma_y^2}{nk^2} = 4\frac{\sigma_y^2}{nk}\lambda'^2 = \sigma_{\lambda'^2}^2.$$

To find the variance of λ' from this last equation we note that

$$\delta\lambda'^2 = \frac{d\lambda'^2}{d\lambda'}\delta\lambda' = 2\lambda'\delta\lambda'$$

and, consequently,

$$\overline{(\delta\lambda'^2)^2} = 4\lambda'^2\overline{(\delta\lambda')^2} = \sigma_{\lambda'^2}^2 = \frac{4\sigma_y^2}{nk}\lambda'^2,$$

giving

$$\overline{(\delta\lambda')^2} = \sigma_{\lambda'}^2 = \frac{\sigma_y^2}{nk}.$$

The variances of λ' and λ, the standard deviations of the different means around the means of the composite distribution, are thus merely the variances of the individual means divided by the number of component populations. Furthermore, since the correlation between averages of samples of n drawn from a single population is the same as the correlation between single measures (Appendix), the correlation between the errors in λ and λ' must also be r, the correlation between single measures in the individual populations. The required variance of m, the slope of the line of relation, is accordingly,

$$\sigma_m^2 = \frac{\sigma_y^2 - 2mr\sigma_x\sigma_y + m^2\sigma_x^2}{nk(S_X^2 - \sigma_x^2)}$$

or

$$\sigma_m^2 = \frac{S_Y^2 - 2mp_{XY} + m^2S_X^2}{N(S_X^2 - \sigma_x^2)},$$

in which S_X^2, S_Y^2 are the respective variances of the two variates of the composite distribution around the means of these variates, and N is the total number of measures.

The form of the last equation shows that the variance of m is a quadratic function of m, that it is independent of the nature of the point set representing the means of the component distributions, and that it is applicable to any linear, composite, bivariate distribution. The preceding equation, on the other hand, shows that the equation giving the variance of the slope of a linear regression line (2) is merely the special case of this equation obtained on writing $\sigma_x = 0$.

References

1. CHARNLEY, F. Can. J. Research, A, 19(12) : 139–151. 1941.
2. FISHER, R. A. Statistical methods for research workers. 5th ed. rev. and enl. Oliver and Boyd, Edinburgh. 1934.

APPENDIX

Let samples of n be drawn from a bivariate population in which two characters X and Y are correlated with correlation r. If averages of the two variates in samples of n are correlated, the means of the two distributions will evidently remain the same. Let deviations be taken from the respective means and let

$$u_1 = \frac{x_{11} + x_{12} + - - + x_{1n}}{n}, \quad v_1 = \frac{y_{11} + y_{12} + - - - + y_{1n}}{n}$$

$$- -$$

$$u_N = \frac{x_{N1} + x_{N2} + - - - + x_{Nn}}{n}, \quad v_N = \frac{y_{N1} + y_{N2} + - - - + y_{Nn}}{n}.$$

Then $\sigma_u = \frac{\sigma_1}{\sqrt{n}}$, $\sigma_v = \frac{\sigma_2}{\sqrt{n}}$, and $\bar{r} = \frac{\Sigma uv}{N\sigma_u\sigma_v}$, where $\bar{r}$ is the correlation between the averages in the samples of n.

Substituting the values of u and v in the product term we have

$$\Sigma uv = u_1v_1 + u_2v_2 + - - - - + u_Nv_N .$$

$$\begin{aligned} n^2\Sigma uv &= (x_{11} + x_{12} + - - - + x_{1n})(y_{11} + y_{12} + - - - + y_{1n}) \\ &\quad + (x_{21} + x_{22} + - - - + x_{2n})(y_{21} + y_{22} + - - - + y_{2n}) \\ &\quad + - - - - - - - - - - - - - - - - - - - \\ &= x_{11}y_{11} + x_{12}y_{12} + - - - + x_{1n}y_{1n} + x_{21}y_{21} + x_{22}y_{22} \\ &\quad + - - - - - - + x_{2n}y_{2n} + - - - + \text{products in which the} \end{aligned}$$

independent variate of a given pair in a sample is associated with the dependent variates of other pairs in the sample. By a slight extension of the product theorem* the sum of the latter must be zero. Hence

$$n^2\Sigma uv = \Sigma xy,$$

where x and y are corresponding values in single samples. Accordingly,

$$\bar{r} = \frac{\Sigma xy}{n^2}\,\frac{n}{N\sigma_1\sigma_2} = \frac{\Sigma xy}{Nn\sigma_1\sigma_2} = r,$$

since the total number of measures is Nn.

*KELLEY T. L. Statistical method. The Macmillan Company, New York. 1923.

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LE CALCIUM ET LE MANGANÈSE DANS LES SÈVES ET LES SIROPS D'ÉRABLE[1]

PAR ELPHÈGE BOIS[2], LOUIS-CHARLES DUGAL[3] et MAURICE LESSARD[4]

Résumé

Au cours de la concentration de la sève d'érable en sirop, il y a précipitation des sels de calcium et de manganèse. Dans les sirops à 63% de solides il y a deux fois plus de CaO que dans ceux à 67%. Dans un évaporateur en aluminium, au moins 75% du manganèse est précipité. C'est là une des causes qui font varier les indices analytiques des sirops filtrés.

Introduction

La sève d'érable contient du sucrose et aussi des sels (6, 7). Au cours de la fabrication du sirop, la concentration dans l'évaporateur provoque la précipitation d'une partie de ces sels. Ceux qui restent dans le sirop filtré ont servi de base à une méthode d'analyse pour reconnaître la falsification des produits de l'érable. Cette méthode procède soit par la mesure de la conductivité électrique du sirop dilué, soit encore par la calcination du sirop et les déterminations des cendres totales, solubles et insolubles, aussi l'alcalinité de ces cendres. Les valeurs ainsi obtenues doivent être comprises entre certaines limites qui ont été fixées par l'analyse d'un grand nombre de sirops de pureté reconnue (3, 9, 10, 11).

Signalons que Delorme (5), Riou et Delorme (8) ont proposé un indice de manganèse.

Nous considérerons la teneur en calcium et en manganèse des sèves et des sirops d'érable, ce qui permettra d'expliquer, en partie du moins, la variation des valeurs limites ou indices analytiques des *sirops filtrés*.

Partie expérimentale

La minéralisation

Pour la détermination de ces éléments, il est nécessaire de détruire la matière organique. Les résultats obtenus par la calcination varient suivant des causes insuffisamment déterminées: la température plus ou moins élevée atteinte durant l'opération et la formation de composés plus ou moins solu-

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bles. La minéralisation par voie humide élimine ces causes. Elle est employée avec succès dans nombre d'analyses biochimiques (2, 4).

Nous avons choisi celle par l'acide nitrique en présence de l'acide sulfurique (1, p. 371); sa conduite est facile avec les sirops et les risques d'accidents sont minimes. Après la destruction de la matière organique (nous prenons une quantité de sève ou de sirop contenant 20 g. de matière solide, d'après l'indice de réfraction), le liquide est porté à 500 ml. et les déterminations des différents éléments se poursuivent sur des aliquots.

Le calcium

Nous avons prélevé 50 ml. de la solution et précipité le calcium sous forme d'oxalate (1, p. 347). Ce dernier a été dosé par une solution *N*/50 de permanganate de potassium. Les résultats sont donnés en pourcentage de CaO par rapport à la matière solide. Voir le Tableau I.

TABLEAU I

LE CALCIUM DANS LES SÈVES ET LES SIROPS D'ÉRABLE

Échantillons	Solides, %	CaO, % de solides
Mélange *A*, sèves *S.E.P.* 1938		0.413
Mélange *B*, sèves *S.E.P.* 1938		0.418
Mélange *C*, sèves *S.E.P.* 1938		0.418
Mélange *A*, sèves *S.E.P.* 1939		0.350
Mélange *B*, sèves *S.E.P.* 1939		0.388
Mélange *C*, sèves *S.E.P.* 1939		0.449
Mélange *A*, sèves *S.E.P.* 1940		0.540
Mélange *B*, sèves *S.E.P.* 1940		0.602
N° 126, sirop *S.E.P.* 1940	63.3	0.502
N° 108, sirop *S.E.P.* 1940	63.8	0.530
N° 115, sirop *S.E.P.* 1940	65.9	0.400
N° 119, sirop *S.E.P.* 1940	67.0	0.221
N° 112, sirop *S.E.P.* 1940	67.0	0.185
N° 123, sirop *S.E.P.* 1940	67.2	0.196
Sirop *M* 1940	66.0	0.115
Sirop *N* 1940	67.2	0.118

Le manganèse

Les déterminations du manganèse furent faites suivant la méthode au persulfate (1, p. 521). Les résultats sont exprimés en parties de manganèse par million de parties de matière solide dans l'échantillon. Voir le Tableau II.

Commentaires et conclusions

Les sèves provenaient de la Station expérimentale de Plessisville, P.Q. Leur teneur en calcium et en manganèse varie sensiblement au cours de la même saison et davantage d'une saison à l'autre. Les sirops *S.E.P.* furent

TABLEAU II

LE MANGANÈSE DANS LES SÈVES ET LES SIROPS D'ÉRABLE

Échantillons	Solides, %	Mn, p.p.m. de solides
Mélange *A*, sèves *S.E.P.* 1940	2.4	22.0
Mélange *B*, sèves *S.E.P.* 1940	2.6	20.0
Mélange *C*, sèves *S.E.P.* 1940	3.1	33.0
N° 110, sirop *S.E.P.* 1940	62·3	1·0
N° 126, sirop *S.E.P.* 1940	63.3	4.3
N° 108, sirop *S.E.P.* 1940	63.8	3.5
N° 112, sirop *S.E.P.* 1940	67.0	1.5
N° 123, sirop *S.E.P.* 1940	67.2	4.0
Sirop *M* 1940	66.0	70.0
Sirop *N* 1940	67.2	63.0

préparés au même endroit dans des ustensiles en aluminium: chalumeaux, chaudières à sève, réservoirs et évaporateur. Les sirops *M* et *N* provenaient d'une érablière voisine munie d'appareils en fer-blanc. Tous les sirops ont été filtrés sur feutre. On constate que le pourcentage de CaO diminue à mesure qu'augmente la concentration des sirops. C'est à 65% de solides environ que la variation dans la concentration du sirop affecte le plus la quantité de CaO. Il y a deux fois plus de CaO dans les sirops à 63% que dans ceux à 67%. Il est à remarquer que la teneur en calcium n'est pas constante pour une concentration donnée du sirop; les sirops à 67.0% et 67.2% de solides en sont des exemples. Nous avons constaté en outre que l'ébullition du sirop d'érable filtré provoque une nouvelle précipitation. La gradation observée pour le calcium ne s'est pas répétée pour le manganèse.

Nous soulignons que Delorme (5, p. 33) a déterminé l'indice de manganèse des sèves de St-Vincent-de-Paul et qu'il a trouvé des valeurs identiques aux nôtres pour les sèves de Plessisville: 20 à 30 p.p.m. (2.0 à 3.0 mg. pour 100 g. de M.S.).

Ces indices, inféreurs à 5, sont ceux de la sève d'érable, le plus pur des produits de l'érable. Les sirops qui ont un indice plus élevé seraient contaminés par le manganèse des ustensiles, comme nos sirops *M* et *N*.

Cette précipitation et cette contamination irrégulière sont au nombre des facteurs qui font varier le pourcentage des cendres, l'alcalinité des cendres, la conductivité électrique, et d'autres indices analytiques des sirops.

Présentement, les déterminations du manganèse ne permettent pas plus que celles du calcium de fixer des limites pour déceler la falsification des produits de l'érable qui ont été *filtrés*.

Bibliographie

1. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official and tentative methods of analysis. 4th ed. A.O.A.C., Washington, D.C. 1935.
2. BARTHOLOMEW, R. J. Analyst, 63 : 884-886. 1938.
3. BRYAN, A. H. U. S. Dept. Agr. Bull. 134. 1910.
4. BUELL, M. V. J. Biol. Chem. 130 (1) : 357-363. 1939.
5. DELORME, J. Rev. Oka, 11 : 3-13, 30-40. 1937.
6. JONES, C. H. Vermont Agr. Expt. Sta. 18th Ann. Rept., pp. 315-339. 1905.
7. JONES, C. H. et HOLLISTER, F. M. Vermont Agr. Expt. Sta. 17th Ann. Rept., pp. 457-461. 1904.
8. RIOU, P. et DELORME, J. Can. J. Research, B, 19 : 68-72. 1941.
9. SNELL, J. F. Ind. Eng. Chem. 5 (9) : 740-747. 1913.
10. SNELL, J. F. Trans. Roy. Soc. Can. (Ser. 3) 13 (Sec. 3) : 221-231. 1919.
11. SNELL, J. F. et SCOTT, J. M. Ind. Eng. Chem. 5 (12) : 993-997. 1913.

CHEMOTHERAPEUTIC STUDIES: PREPARATION OF SUBSTITUTED SULPHONAMIDES[1]

By C. Marchant[2], C. C. Lucas[3], and L. McClelland[2]

WITH AN APPENDIX

THE DETERMINATION OF CHEMOTHERAPEUTIC ACTIVITY

By P. H. Greey[4]

Abstract

A series of substituted sulphonamides has been prepared for a study of their chemotherapeutic activity against a number of different bacterial species. By condensing *p*-acetylaminobenzenesulphonyl chloride with primary aromatic and aliphatic amines, N^4-acetyl-N^1-substituted sulphanilamides were obtained. Hydrolysis with acid or alkali removed the acetyl group and gave the desired N^1-substituted sulphanilamides.

The appendix contains details of the techniques used in testing these sulphonamides. Although a few of the compounds described in this paper exhibit considerable *in vitro* activity, none of them possess, *in vivo*, chemotherapeutic properties equal to those of sulfapyridine or sulfathiazole.

Introduction

Late in 1937 this laboratory became interested in the biochemical, clinical, and experimental aspects of the chemotherapy of bacterial infections. The results of these studies (5, 7, 8, 9) so stimulated the writers' interest that a program of synthesis was embarked upon, in the hope of finding drugs with specific activity towards staphylococci, *M. tuberculosis*, or other species not controlled by sulphanilamide.

Since the latter part of 1938 a large number of new sulphonamides have been prepared by the writers and tested by Dr. P. H. Greey, of the Department of Pathology and Bacteriology, against many different bacterial species *in vitro* and against *Streptococcus haemolyticus*, *Pneumococcus*, and *Staphylococcus in vivo;* furthermore, the curative power of some was tested with guinea pigs infected with *M. tuberculosis*. Several of these derivatives were fairly active against streptococci, but they were not as good as sulphanilyl α-amino pyridine (*M* and *B* 693, dagenan, sulfapyridine). Although no new drug with particularly desirable antibacterial characteristics has been found, it seems advisable to put on record some of the substances studied to date. A few of the compounds prepared and tested here have, in the meantime, been examined in other laboratories and are now recorded in the literature; the writers have learned that certain others have been studied elsewhere, but the findings have never been published.

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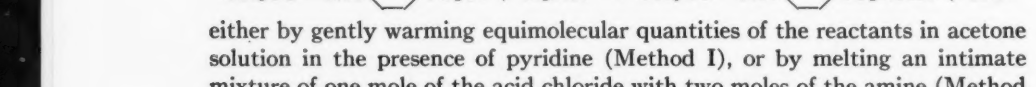
The accompanying list of compounds is submitted in the hope that others may also record compounds tested and found to be unsuitable, since only in this way can unnecessary duplication of effort be avoided. The writers know of one case in which at least five different laboratories studied the same compound, but none of them published their negative findings. More rapid advances could undoubtedly be made if the present wasteful replication of work could be eliminated.

The substituted sulphanilamides listed in this publication were prepared by condensing *p*-acetylaminobenzenesulphonyl chloride with an amine (see equation)



either by gently warming equimolecular quantities of the reactants in acetone solution in the presence of pyridine (Method I), or by melting an intimate mixture of one mole of the acid chloride with two moles of the amine (Method II). The product of the reaction in acetone was precipitated by the addition of four volumes of cold water. This impure material, or the product obtained by the fusion procedure, was purified by dissolving in a minimum quantity of cold dilute sodium hydroxide solution, extracting with ether to remove impurities, precipitating from the alkaline solution with dilute hydrochloric acid, and recrystallizing from a suitable solvent. The yields of the pure compounds ranged from 50 to 95%, but were usually more than 70%. Some properties of the compounds so obtained are shown in Table I. Attempts to condense arsanilic acid with *p*-acetylaminobenzenesulphonyl chloride were completely unsuccessful. N^4-acetyl-N^1-(4-arsonophenyl)-sulphanilamide was finally obtained by the Bart reaction (1) from N^4-acetyl-N^1-(4-aminophenyl)-sulphanilamide.

To remove the acetyl radical protecting the amino group, the compounds were hydrolysed by refluxing with either hydrochloric acid or sodium hydroxide solution. The product of the acid hydrolysis, which usually separated out on cooling, was recrystallized from aqueous ethyl alcohol or other suitable solvent. When alkaline hydrolysis was employed the product was obtained by neutralizing to litmus, filtering, and recrystallizing. The yields, based on the acetyl derivative taken, were between 50 and 90%. The compounds so prepared are listed in Table II.

Compounds containing a nitro group were usually converted to the corresponding amino compound by catalytic reduction. Theoretical amounts of hydrogen were taken up, but, owing to losses in purification, the final yield of pure product was usually only 50 to 75% of that expected from the nitro compound. In a few cases, reduction with ferrous hydroxide was used.

The carbethoxy compounds were made in the usual way by esterifying the acids with absolute ethyl alcohol in the presence of hydrogen chloride. The acids with carboxyl groups para or meta to the sulphonamide linkage esterified readily, but the ortho compound gave only low yields even with much longer refluxing. These compounds are listed in Table II.

TABLE I

N^4-ACETYL-N^1-SUBSTITUTED SULPHANILAMIDES

N^4-Acetyl-N^1-substituted sulphanilamide	M.p.° C.†	Method of prepn.	Crystal form	Recrystallized from
N^1-(4-nitrophenyl) (2, 11)	258	I, II	Light yellow rods	95% Alcohol
N^1-(3-nitrophenyl) (11)	244	I	Light yellow prisms	50% Alcohol
N^1-(2-nitrophenyl) (11)	200	II	Yellow prisms	95% Alcohol
N^1-(6-methyl-3-nitrophenyl)	266.5	I	Light yellow plates	50% Acetone
N^1-(4-methyl-3-nitrophenyl)	239	I	Light yellow needles	95% Alcohol
N^1-(6-methoxy-3-nitrophenyl)	261.5 dec.	I	Light yellow plates	1 *M* Sodium carbonate
N^1-(4-methoxy-2-nitrophenyl)	175	I	Light yellow rods	50% Alcohol
N^1-(4-aminophenyl)	235		Colourless needles	50% Alcohol
N^1-(4-methylphenyl)	208	I	Colourless hexagonal rods	95% Alcohol
N^1-(3-methylphenyl)	205	I	Colourless pyramids	50% Alcohol
N^1-(2-methylphenyl)	244.5	I	Colourless prisms	95% Alcohol
N^1-(4-methoxyphenyl)	200	I, II	Colourless plates	95% Alcohol
N^1-(3-methoxyphenyl)	193	I	Colourless pyramids	95% Alcohol
N^1-(2-methoxyphenyl)	212	I	Colourless plates	95% Alcohol
N^1-(4-carboxyphenyl)* (2, 6)		I	——	——
N^1-(3-carboxyphenyl) (6)	274	I	Colourless rods	50% Alcohol
N^1-(2-carboxyphenyl) (6)	233	I	Colourless plates	75% Alcohol
N^1-(2, 6-dimethylphenyl)	236.5	I	Colourless flat rods	95% Alcohol
N^1-(2, 4-dimethylphenyl)	214.5	I	Colourless needles	95% Alcohol
N^1-(2-methoxy-5-methylphenyl)	206	II	Colourless rods	95% Alcohol
N^1-(2-methyl-5-isopropylphenyl)	160.5	I	Colourless prisms	95% Alcohol
N^1-(4-acetylphenyl)	254.5	I	Colourless plates	95% Alcohol
N^1-(4-benzoylphenyl)	218.5	I	Colourless rods	95% Alcohol
N^1-(4-bromophenyl)	208	I	Colourless rods	95% Alcohol
N^1-(3-hydroxyethyl) (2)	150	II	Colourless plates	Water
N^1-(4-arsonophenyl)	275 dec.		Colourless rods	30% Alcohol
bis-(acetyl-sulphanilyl)-*p*-phenylene-diamine	316.5 dec.	I	Colourless plates	1 *M* Sodium carbonate
bis-(acetyl-sulphanilyl)-*m*-toluylene-diamine	278	I	Colourless rods	95% Alcohol
bis-(acetyl-sulphanilyl)-benzidine	288	I	Colourless rods	Glacial acetic

* *Prepared but not isolated as pure chemical.*

† *All melting points are corrected.*

Most of these compounds were analysed, but, since the de-acetylated forms derived from them were of correct composition (Table II), the analytical data are not given here.

The method of testing the chemotherapeutic activity of the compounds listed in Table II is briefly described by Dr. Greey in an appendix to this paper. The intermediate compounds shown in Table I were not tested, since many workers (3) have shown that the N^4-substituted derivatives of compounds known to be active possess a greatly diminished activity.

Experimental

p-Acetylaminobenzenesulphonyl chloride. A modification of the method described by Stewart (10) was used to prepare this compound. To 466 gm. (4 moles) of technical chlorosulphonic acid was added 135 gm. (1 mole) of acetanilide, in small portions, with vigorous stirring, care being taken that the temperature did not rise above 70° C. The resulting solution was main-

TABLE II

N^1-SUBSTITUTED SULPHANILAMIDES

Ref. No.	N^1-substituted sulphanilamide	Formula	M.p.°C. †	N %††		Crystal form	Recrystallized from
				Calc.	Found		
1	N^1-(4-nitrophenyl) (2, 11)	$C_{12}H_{11}N_3O_4S$	165	—	—	Light yellow rods	95% Alcohol
2	N^1-(3-nitrophenyl) (11)	$C_{12}H_{11}N_3O_4S$	169	—	—	Yellow rods	50% Alcohol
3	N^1-(2-nitrophenyl)	$C_{12}H_{11}N_3O_4S$	179	—	—	Yellow rods	50% Alcohol
4	N^1-(6-methyl-3-nitrophenyl)	$C_{13}H_{13}N_3O_4S$	199	—	—	Yellow rods	95% Alcohol
5	N^1-(4-methyl-3-nitrophenyl)	$C_{13}H_{13}N_3O_4S$	189	—	—	Light yellow rods	95% Alcohol
6	N^1-(6-methoxy-3-nitrophenyl)	$C_{13}H_{13}N_3O_5S$	188	—	—	Yellow plates	95% Alcohol
7	N^1-(4-methoxy-2-nitrophenyl)	$C_{13}H_{13}N_3O_5S$	117	—	—	Yellow rods	95% Alcohol
8	N^1-(4-aminophenyl) (2, 11)	$C_{12}H_{13}N_3O_2S$	138	15.95	15.75	Colourless needles	25% Alcohol
9	N^1-(3-aminophenyl) (11)	$C_{12}H_{13}N_3O_2S$	177	15.95	15.97	Colourless plates	50% Alcohol
10	N^1-(2-aminophenyl) (11)	$C_{12}H_{13}N_3O_2S$	208	15.95	16.00	Colourless needles	95% Alcohol
11	N^1-(6-methyl-3-aminophenyl)	$C_{13}H_{15}N_3O_2S$	208.5	15.16	15.06	Colourless plates	75% Alcohol
12	N^1-(4-methyl-3-aminophenyl)	$C_{13}H_{15}N_3O_2S$	185	15.16	15.17	Colourless plates	50% Alcohol
13	N^1-(6-methoxy-3-aminophenyl)	$C_{13}H_{15}N_3O_3S$	232	14.32	14.13	Colourless plates	70% Alcohol
14	N^1 (4-methoxy-2-aminophenyl)	$C_{13}H_{15}N_3O_3S$	195	14.32	14.43	Colourless needles	60% Alcohol
15	N^1-(4-methylphenyl)	$C_{13}H_{14}N_2O_2S$	190	10.68	10.32	Colourless rods	95% Alcohol
16	N^1-(3-methylphenyl)	$C_{13}H_{14}N_2O_2S$	133.5	10.68	10.45	Colourless rods	50% Alcohol
17	N^1-(2-methylphenyl)	$C_{13}H_{14}N_2O_2S$	155.5	10.68	10.73	Colourless flat rods	95% Alcohol
18	N^1-(4-methoxyphenyl)	$C_{13}H_{14}N_2O_3S$	194	10.06	9.97	Colourless rods	95% Alcohol
19	N^1-(3-methoxyphenyl)	$C_{13}H_{14}N_2O_3S$	163.5	10.06	10.10	Colourless rods	95% Alcohol
20	N^1-(2-methoxyphenyl)	$C_{13}H_{14}N_2O_3S$	199	10.06	9.94	Colourless prisms	50% Alcohol
21	N^1-(4-carboxyphenyl) (2, 4, 6)	$C_{13}H_{12}N_2O_4S$	197	9.62	9.61	Colourless rods	Water
22	N^1-(3-carboxyphenyl) (6)	$C_{13}H_{12}N_2O_4S$	196	9.62	9.49	Colourless plates	40% Alcohol
23	N^1-(2-carboxyphenyl) (4, 6)	$C_{13}H_{12}N_2O_4S$	226	9.62	9.56	Colourless rods	75% Alcohol
24	N^1-(4-carbethoxyphenyl)	$C_{15}H_{16}N_2O_4S$	230	8.75	8.66	Colourless plates	30% Alcohol
25	N^1-(3-carbethoxyphenyl)	$C_{15}H_{16}N_2O_4S$	105	8.75	8.68	Colourless rods	95% Alcohol
26	N^1-(2-carbethoxyphenyl)	$C_{15}H_{16}N_2O_4S$	165.5	8.75	8.79	Colourless rods	30% Alcohol
27	N^1-(2, 6-dimethylphenyl)	$C_{14}H_{16}N_2O_2S$	231	10.13	10.15	Colourless needles	95% Alcohol
28	N^1-(2, 4-dimethylphenyl)	$C_{14}H_{16}N_2O_2S$	149	10.13	10.18	Colourless rods	80% Alcohol
29	N^1-(2-methoxy-5-methylphenyl)	$C_{14}H_{16}N_2O_3S$	161	9.58	9.56	Colourless plates	50% Alcohol
30	N^1-(2-methyl-5-isopropylphenyl)	$C_{16}H_{20}N_2O_2S$	150.5	9.19	9.10	Colourless plates	50% Alcohol
31	N^1-(4-acetylphenyl)	$C_{14}H_{14}N_2O_3S$	211	9.65	9.68	Colourless rods	95% Alcohol
32	N^1-(4-benzoylphenyl)	$C_{19}H_{16}N_2O_3S$	181.5	7.95	7.94	Colourless needles	95% Alcohol
33	N_1-(4-bromophenyl)	$C_{12}H_{11}N_2O_2SBr$	178	8.56	8.58	Colourless plates	95% Alcohol
34	N^1-β-hydroxyethyl (2)	$C_8H_{12}N_2O_3S$	100	12.95	12.94	Colourless plates	Water
35	N^1-(4-arsonophenyl)*	$C_{12}H_{12}N_2O_6SAs$	**	7.53	7.50	Colourless rods	Water
36	bis-(sulphanilyl)-*p*-phenylenediamine	$C_{18}H_{18}N_4O_4S_2$	263 dec.	13.39	13.51	Colourless plates	1 *M* Sodium carbonate
37	bis-(sulphanilyl)-*m*-toluylenediamine	$C_{19}H_{20}N_4O_4S_2$	229	12.95	12.94	Colourless needles	95% Alcohol
38	bis-(sulphanilyl)-benzidine	$C_{24}H_{22}N_4O_4S_2$	293	11.33	11.28	Colourless plates	1 *M* Sodium carbonate

* *% As. calc. 20.14, found 20.27.*
† *All melting points are corrected.*
** *Chars between 241° C. and 250° C.*
†† *Determined by Kjeldahl method.*

tained at 60° to 65° C. for two hours then poured slowly with vigorous stirring on to 1 kg. of crushed ice. The next steps were carried out in quick succession, since the compound hydrolyses somewhat readily while moist with the acid mother liquor. The precipitate was filtered with suction, washed with 200 cc. of cold water, and partly dried by suction. The paste so obtained was dis-

solved in about 500 cc. of acetone and precipitated by the addition of two to three litres of cold water, filtered off with suction, and dried in the air. This product (yield, 130 to 140 gm., i.e., 55 to 60%), m.p. above 140° C*., was sufficiently pure to be used in the preparations described below. When kept dry it remained relatively stable for more than a month. Partly decomposed material could be purified by reprecipitation from acetone with water.

Preparation of N^4-Acetyl-N^1-substituted Sulphanilamides

Method I

This method, which involves the use of pyridine as condensing agent, has been described previously by Bauer (2), but none of the preparations given below were reported by him.

N^4-Acetyl-N^1-(4-methoxyphenyl)-sulphanilamide. A solution of 23.4 gm. (0.1 mole) of *p*-acetylaminobenzenesulphonyl chloride, 12.3 gm. (0.1 mole) of *p*-anisidine and 12 cc. (about 0.15 mole) of technical pyridine in 80 cc. of acetone was brought just to the boil and allowed to cool to room temperature by standing for two hours. The product, after precipitating by adding about 350 cc. of cold water and filtering, was dissolved in a minimum quantity of 5 *N* sodium hydroxide solution. The solution was diluted with water to about three times the volume, shaken with an equal volume of ether to remove any pyridine and unused amine, and the product was precipitated with dilute hydrochloric acid. The resulting material was dissolved in hot 95% ethyl alcohol, decolorized with charcoal, and allowed to crystallize. On recrystallizing again, 21.4 gm. (70% yield) of the pure compound was obtained as colourless plates, m.p. 200° C. When prepared by Method II, the yield was 24.5 gm. (80%).

N^4-Acetyl-N^1-(6-methoxy-3-nitrophenyl)-sulphanilamide. A solution of 23.4 gm. (0.1 mole) of *p*-acetylaminobenzenesulphonyl chloride, 16.8 gm. (0.1 mole) of 6-methoxy-3-nitro-aniline and 12 cc. (about 0.15 mole) of technical pyridine in 80 cc. of acetone was brought just to the boil and allowed to cool to room temperature by standing for two hours. The product was precipitated by adding about 350 cc. of cold water and purified as described above, except that it was recrystallized from a 1 *M* solution of sodium carbonate. The compound was obtained as light yellow plates, m.p. 261.5° C., dec. Yield, 27.5 gm. (75%).

N^4-Acetyl-N^1-(4-methyl-3-acetylsulphanilamido-phenyl)-sulphanilamide.† A solution of 46.8 gm. (0.2 mole) of *p*-acetylaminobenzenesulphonyl chloride, 12.4 gm. of *m*-toluylenediamine, 24 cc. (about 0.3 mole) of technical pyridine in 160 cc. of acetone was brought just to the boil and allowed to cool to room temperature by standing for two hours. The product was precipitated by

* *The pure product, recrystallized from benzene, melted at 149° C.*
All melting points are corrected.

† *A shorter name is used in Table I for this compound, viz., bis-(acetylsulphanilyl) m-toluylenediamine.*

adding 350 cc. of cold water and purified by recrystallization from 95% ethyl alcohol; yield, 28 gm. (60%), as colourless rods, m.p. 278° C.

N^4-Acetyl-N^1-(4-carboxyphenyl)-sulphanilamide. A solution of 23.4 gm. (0.1 mole) of *p*-acetylaminobenzenesulphonyl chloride, 13.7 gm. (0.1 mole) of *p*-aminobenzoic acid, and 12 cc. (about 0.15 mole) of technical pyridine in 80 cc. of acetone was brought just to the boil and allowed to cool. After two hours the product was precipitated by adding 350 cc. of cold water. The filtered material was washed with 100 cc. of warm 5 *N* hydrochloric acid to remove any pyridine and unused amine. The insoluble remainder was dissolved in a minimum volume of hot 5 *N* sodium hydroxide solution and cooled. The sodium salt of the compound, which precipitated, was dissolved in hot 50% ethyl alcohol, decolorized with charcoal, and allowed to crystallize. The free acid was obtained by dissolving the sodium salt in water and precipitating with dilute hydrochloric acid. The pure compound was obtained from 30% ethyl alcohol as colourless plates, m.p. 253° C., yield, 22.0 gm. (66%).

Method II

This method, involving fusion of the reactants, gives good yields only when the fusion temperature is not too high and when the fusion mass is not too viscous.

N^4-Acetyl-N^1-(2-methoxy-5-methylphenyl)-sulphanilamide. An intimate mixture of 23.4 gm. (0.1 mole) of *p*-acetylaminobenzenesulphonyl chloride and 27.4 gm. (0.2 mole) of 2-methoxy-5-methylaniline was melted on a water-bath with vigorous stirring, and heated until solid. The product was isolated by dissolving in a minimum quantity of 5 *N* sodium hydroxide solution, diluting with water to three times the volume, and shaking with an equal volume of ether to remove the excess amine, then precipitating with dilute hydrochloric acid. The resulting material was purified by dissolving in hot 95% ethyl alcohol, decolorizing with charcoal, and allowing to crystallize. On recrystallization, 21.7 gm. (65% yield) of colourless rods (m.p. 206° C.) was obtained.

N^4-Acetyl-N^1-(2-nitrophenyl)-sulphanilamide. An intimate mixture of 23.4 gm. (0.1 mole) of *p*-acetylaminobenzenesulphonyl chloride and 27.6 gm. (0.2 mole) of *o*-nitraniline was melted on a water-bath to a liquid and stirred vigorously until almost solid. The product was purified as described above. A yield of 20.0 gm. (60%), as bright yellow prisms (m.p. 200° C.), was obtained. Attempts to prepare this by Method I were not successful.

N^4-Acetyl-N^1-β-hydroxyethyl-sulphanilamide. A solution of 23.4 gm. (0.1 mole) of *p*-acetylaminobenzenesulphonyl chloride in 12.1 gm. (0.2 mole) of ethanolamine was warmed on a water-bath for one hour. After cooling, the precipitated material was filtered off and recrystallized twice from water, being decolorized with charcoal the first time. The compound was obtained as colourless plates (m.p. 150° C.) in 90% yield (23.3 gm.).

Removal of the N^4-Acetyl Group

Both acid and alkali have been used to hydrolyse these compounds. Since the acetylated compounds are usually insoluble in the acid, the early stages of hydrolysis were accompanied by frothing and bumping. To avoid this, in all later work alkaline hydrolysis was employed, since the sodium salt of these compounds is water-soluble.

Acid Hydrolysis

N^1-(4-Methoxyphenyl)-sulphanilamide. This compound was obtained by refluxing 32.0 gm. (0.1 mole) of N^4-acetyl-N^1-(4-methoxyphenyl)-sulphanilamide with 160 cc. of 5 *N* hydrochloric acid until completely dissolved. This required one hour. On cooling, the hydrochloride of the compound precipitated. Recrystallization from 95% ethyl alcohol gave the free amine. On recrystallizing once more, 22.0 gm. (80% yield based on the acetylated compound) of colourless rods (m.p. 194° C.) was obtained.

Alkaline Hydrolysis

Bis-sulphanilyl-m-toluylene-diamine. A solution of bis-acetylsulphanilyl-*m*-toluylene-diamine in 125 cc. of 2 *N* sodium hydroxide* solution was refluxed for one hour. After cooling, the reaction product was precipitated by neutralizing to litmus with dilute hydrochloric acid and recrystallized twice from 95% ethyl alcohol; colourless needles, m.p. 229° C. Yield, 30.0 gm. (70%, based on acetylated compound).

Reduction of Nitro Group to Amino Group

N^1-(6-Methoxy-3-aminophenyl)-sulphanilamide. A suspension of 3.2 gm. (0.01 mole) of N^1-(6-methoxy-3-nitrophenyl)-sulphanilamide in 30 cc. of 95% ethyl alcohol was shaken with palladium black in an atmosphere of hydrogen at room temperature and atmospheric pressure; the theoretical volume of hydrogen was taken up over a period of three hours. The colour of the suspension changed from yellow to colourless. The amine was dissolved in the alcohol by warming, the palladium filtered off, and the product crystallized from the hot filtrate suitably diluted with water. The amine, which discolours very readily when exposed to air, was dissolved in 70% ethyl alcohol; the solution was treated with charcoal and filtered, and the amine allowed to crystallize. After this operation had been repeated, the yield of pure compound as colourless plates (m.p. 232° C.), was 1.1 gm. (52%). The fact that the theoretical quantity of hydrogen was taken up suggested that the losses occurred during purification. By converting the amine to the hydrochloride and dissolving in hot water, the colour was removed more readily with charcoal and the yield could be increased to 90%. The greater stability of the hydrochloride is a further advantage of this procedure.

** When a carboxy- or arsono-substituted compound was hydrolysed sufficient additional sodium hydroxide was used in order to form the sodium salt.*

N^1-(2-Aminophenyl)-sulphanilamide. A solution of 3.1 gm. (0.01 mole) of the sodium salt of N^1-(2-nitrophenyl)-sulphanilamide in water was stirred vigorously for 15 min. with a suspension of ferrous hydroxide prepared by dissolving 20 gm. of technical ferrous sulphate in 50 cc. of water and making alkaline with about 90 cc. of 2 *N* sodium hydroxide solution. The mixture was filtered through a large suction filter and the ferric hydroxide extracted twice with about 50 cc. of water. The crude product was obtained from the combined washings and filtrate by acidifying with acetic acid and filtering. The yield after decolorizing and recrystallizing from 95% ethyl alcohol was 1.8 gm. (70%). This method has been used by Webster and Powers (11).

Esterification of Carboxyl Groups

N^1-(4-Carbethoxyphenyl)-sulphanilamide. To a solution of 2.92 gm. (0.01 mole) of N^1-(4-carboxyphenyl)-sulphanilamide in 25 cc. of absolute alcohol was added 15 cc. of absolute alcohol saturated with dry hydrogen chloride at 0° C. The solution was refluxed for six hours and then cooled; 400 cc. of 0.5 *M* sodium carbonate solution was cautiously added, the mixture was filtered with suction, and the product recrystallized from 50% alcohol. Colourless plates, m.p. 235° C., were obtained in a yield of 2.7 gm. (82%).

Introduction of Arsono Group

This is an application of the Bart reaction (1).

N^4-Acetyl-N^1-(4-arsonophenyl)-sulphanilamide. A fine suspension of the hydrochloride of N^4-acetyl-N^1-(4-aminophenyl)-sulphanilamide* was made by dissolving 30.5 gm. (0.1 mole) in 60 cc. of hot concentrated hydrochloric acid and quickly adding 100 gm. of crushed ice. The diazonium salt was prepared from this by adding 8 gm. of sodium nitrite in 20 cc. of water and stirring for one-half hour until a creamy precipitate remained. The diazonium mixture was made just alkaline to Congo red by addition of sodium hydroxide solution. To this was added 23.5 gm. of arsenious oxide dissolved in 240 cc. of 2 *N* sodium hydroxide solution and a little copper powder as a catalyst. After stirring vigorously at room temperature for two hours no more nitrogen was evolved and the precipitate was completely dissolved. A brown gummy precipitate that formed on neutralization (litmus) was discarded. The addition of more acid and gentle warming removed the last of the gummy material. On cooling, a colourless crystalline precipitate was formed. Recrystallization from 30% ethyl alcohol gave colourless rods (m.p. 275° C., dec.) in 50% yield (20.5 gm.).

Acknowledgment

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* *Prepared by catalytic reduction of N^4-acetyl-N^1-(4-nitrophenyl)-sulphanilamide.*

References

1. BART, H. Ann. 429 : 55-103. 1922.
2. BAUER, H. J. Am. Chem. Soc. 61 (3) : 613-616. 1939.
3. BUTTLE, G. A. H., GRAY, W. H., and STEPHENSON, D. Lancet, 230 : 1286-1290. 1936.
4. CROSSLEY, M. L., NORTHEY, E. H., and HULTQUIST, M. E. J. Am. Chem. Soc. 60 (9) : 2217-2222. 1938.
5. GREEY, P. H., MACLAREN, D. B., and LUCAS, C. C. Can. Med. Assoc. J. 40 (4) : 319-324. 1939.
6. KOLLOFF, H. G. J. Am. Chem. Soc. 60 (4) : 950-951. 1938.
7. LUCAS, C. C. Can. Med. Assoc. J. 39 (2) : 111-114. 1938.
8. LUCAS, C. C. and MITCHELL, D. R. Can. Med. Assoc. J. 40 (1) : 27-34. 1939.
9. MITCHELL, D. R., GREEY, P. H., and LUCAS, C. C. Can. Med. Assoc. J. 40 (4) : 336-342. 1939.
10. STEWART, J. J. Chem. Soc. 121 : 2555-2561. 1922.
11. WEBSTER, G. L. and POWERS, L. D. J. Am. Chem. Soc. 60 (7) : 1553-1555. 1938.

APPENDIX

THE DETERMINATION OF CHEMOTHERAPEUTIC ACTIVITY

BY PHILIP H. GREEY

The importance of the many variable factors involved in biological experiments is illustrated by the difficulties encountered in testing new compounds for chemotherapeutic activity. For example, in the *in vitro* bacteriostatic tests the composition and temperature of the culture media, size of inoculum, and time of making the observation are important; the variable susceptibility of hosts, virulence of bacterial strains used, size of infecting dose of bacteria, time interval between infection and first treatment, size and frequency of doses given, duration of medication, and other factors affect the *in vivo* results. Without some mention of these points in a report, it is difficult to compare the assessments of activity performed in different laboratories.

Numerous preliminary experiments were necessary to evolve a technique suitable for this study. For this purpose, compounds of known chemotherapeutic activity, such as sulphanilamide and sulfapyridine, were used. The experimental conditions that most clearly demonstrated the bacteriostatic activity of these compounds against various species of bacteria were determined.

I. In Vitro Studies

Stock cultures of a number of recently isolated strains of seven bacterial species were maintained on appropriate solid media. Each compound was tested against several strains of the various species, and each experiment included a known chemotherapeutic agent, usually sulfapyridine, for comparison and also to serve as a control that the conditions of the experiment were such as to demonstrate bacteriostasis.

The *in vitro* studies were performed by adding weighed amounts of dry-sterilized compound to a known volume of sterile Hartley's broth under aseptic conditions, to give a 100 mg. % solution from which the following

dilutions were prepared: 50, 25, 10, 5, 1, 0.1 mg. %. These were dispensed in 5 cc. amounts in sterile test tubes. After 24 hr. incubation any contaminated tubes were rejected and the remainder were inoculated with the test organism. The drug-broth cultures and appropriate controls were incubated at 37° C. and readings made 24 and 48 hr. after inoculation. Bacteriostatic activity was gauged by comparing the opacity produced by the growth in the control and drug-broth tubes. The lowest concentration of drug that showed a marked inhibition of growth when compared with the control was taken as the index of bacteriostatic activity.

The following plan of testing was ultimately adopted:

Strep. haemolyticus—One loopful of a 1 : 1000 dilution of a 24 hr. broth-culture to 5 cc. of drug-broth medium containing 0.20 cc. of sterile horse or ox serum.

Strep. viridans—One loopful of 1 : 1000 dilution of a 24 hr. broth-culture to 5 cc. of plain drug-broth medium.

Pneumococcus—One loopful undiluted 24 hr. broth-culture to 5 cc. of drug-broth medium containing 0.20 cc. of sterile horse or ox serum.

Staph. aureus, *B. coli*, *B. typhosus*, *B. proteus*—One loopful of 1 : 1,000,000 dilution of a 24 hr. broth-culture to 5 cc. of plain drug-broth medium. In the case of *Staph. aureus*, if marked activity was noted in plain broth, a repeat experiment was carried out using the drug-broth with added serum as described above.

II. In Vivo Studies

In vivo experiments were conducted solely with white mice except for the *M. tuberculosis* infections, which were studied in guinea pigs. In preliminary experiments the acute toxicity of the compounds was determined by giving to pairs of animals the following doses of the drugs, dissolved or suspended in 5% gum acacia: 5, 25, 100, and 200 mg. If any of these animals died, the maximum tolerated dose was determined more accurately by repeating the procedure at smaller dosage intervals with larger groups of animals. The figures given in Table III, under the heading "acute toxicity" are, first, the largest test dose causing no deaths, and, second, the smallest test dose that caused all the animals to die. No attempt was made to determine the resulting concentrations of drug in the blood, so no data are available as to how much absorption of the compounds actually occurred. The size of the individual doses used in the *in vivo* tests was usually about one-twelfth the maximum tolerated dose, or if this proved to be more than 10 mg., only 10 mg. was given. (In some cases where no *in vitro* activity was observed, the *in vivo* tests were not done.) The drugs suspended in gum acacia were then administered, by stomach tube, according to the following plan:

First day—at time of infection, 3, 6, and 10 hr. later.

Second, third, and fourth days—at 9 a.m., 1, 5, and 11 p.m.

Fifth, sixth, and seventh days—at 9 a.m., 1, and 5 p.m.

TABLE III

ANTIBACTERIAL ACTIVITY OF THE COMPOUNDS PREPARED

Compound No.	Acute toxicity, mg. per mouse	*In vitro**				*In vivo***			
		Strep.	Pneu.	Staph.	B. Coli	Strep.	Pneu.	Staph.	M. Tb.
1	> 50 <100	+++	+++	++	++	++	0	0	0
2	>400	++	+++		+		0		0
3	>200	0	0	0	0		0	+	
4	>200					++	+		
5	>200					++	0	0	
6	>200	0	0	0	0		0		
7	>100 <150	0			0		0		
8	>200	++	+	0	+	++	0	++	0
9	>200	+	+	0	+		+	0	
10	>200	+	+	+	0	±	0		
11	>200						0		
12	>200					+	0	±	
13	>150 <200						0		
14	>200					+	0	0	
15	>200	+	+	0	0		+		±
16	>400	+	+++		0		0	0	0
17	>400	++	+++		0		+	0	0
18	>200	+	+	0	0		0		0
19	>400	0	++		0		0	0	0
20	>200	0	0	0	0		0		
21	>400	0	0		0		+		0
22	>200	++	+	+	0	±	0		
23	>150 <200	++	+++	++	0	+	+	±	
24	>200						0	+	
25	>200						0		
26	>200						0		
27	>200					0	0		
28	>200	0	0	0	0		+		
29	>400	0	+		0		0		0
30	>200	0	0	0	0		0		
31	>200	0	+	0	+		0	±	
32	>200	0	0	0	0	±	0		
33	>200	0	0	0	0		0		
34	>200	+	+	0	0		0		0
35	>200		0	0	+		0	0	
36	>200	0	0	0	0		0		
37	>200	0	0	0	0	+	0		
38	>200					+	0		
S†		++++	+++	±	++	++++	+	±	+
SP		++++	++++	±	+++	++++	++++	++	+
ST		++++	++++	+++	++++	++++	++++	+++	
SD		++++				++++	++++	+++	

* *The relative activity is expressed by symbols with the following significance:*
++++ *bacteriostatic at concentrations less than 10 mg. per 100 cc.*
+++ *bacteriostatic at concentrations less than 25 mg. per 100 cc.*
++ *bacteriostatic below 50 mg. per 100 cc.*
\+ *bacteriostatic below 100 mg. per 100 cc.*
0 *not bacteriostatic at 100 mg. per 100 cc.*

** ++++ *at least 75% survivors.*
+++ *at least 40% survivors.*
++ *definite prolongation of survival time.*
\+ *slight prolongation of survival time.*

† *S = sulphanilamide.*
SP = sulfapyridine.
ST = sulfathiazole.
SD = sulfadiazine.

Eighth and ninth days—at 9 a.m. and 5 p.m.

Tenth day—at 9 a.m.

A small group of normal (uninfected) animals received the same doses of drug as the treated animals. These served as "chronic toxicity" controls.

Experimental infections were produced in groups of 10 mice as follows:

Strep. haemolyticus

The mouse-virulent strain C203 was used. The organism was grown for 18 hr. in blood-broth directly from the heart's blood of a passage-mouse. This culture was diluted to 1 : 500,000 and 0.5 cc. injected intraperitoneally.

Pneumococcus

A Neufeld strain of *Pneumococcus* Type I was used. An 18 hr. broth-culture made from the heart's blood of a passage-mouse was diluted in broth to 1 : 5,000,000 and 0.5 cc. injected intraperitoneally.

Staph. aureus

The pathogenicity for mice of a number of strains isolated from patients in the wards of the Toronto General Hospital was determined and the strain (Kutt) possessing the greatest virulence selected for use. A culture, isolated the day before, from the heart's blood of a passage-mouse was used to plant blood-agar slopes. The growth on the slope after 20 hr. incubation was washed off with 6 cc. of Hartley's broth. Infection was initiated by the intravenous route, 0.2 cc. of the dense staphylococcal suspension being given to each mouse.

Each lot of compounds tested *in vivo* included a known chemotherapeutic agent—sulphanilamide, sulfapyridine, or sulfathiazole—so that the relative therapeutic merits of the compounds could be compared under identical conditions. Treated animals were kept under observation for 30 days. Mice dying despite treatment were autopsied and suitable cultures made to determine the cause of death.

Mycobacterium tuberculosis

Multicoloured guinea pigs weighing about 350 gm. were infected subcutaneously in the right thigh with 0.2 cc. of a suspension of human tubercle bacilli. This suspension was so prepared that each oil-immersion field contained approximately five acid-fast bacilli. Drug therapy was begun the day before infection was initiated and was continued daily thereafter until death of the animal. The drugs were given by mouth; with the aid of a nasal speculum an appropriate number of small tablets (25 mg.) were dropped at the back of the throat. Four doses (250 mg. per kg.) were given each day, at 9 a.m., 1, 5, and 11 p.m.

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